010SCA05

CT Stem Cell Research Proposal

Title of Project: **The Role of Dormant Replication Origins in Ensuring Genome Integrity in Human Embryonic Stem Cells**
Amount requested: $200,000; Amount funded: $200,000
Principal Investigator**: XinQuan Ge, PhD**
Institution: **Yale University**

This Project's purpose is to investigate the role of dormant replication origins in maintaining the genome integrity of hESCs during their self-renewal and differentiation.

Project Summary

Maintenance of genome integrity is especially important for stem cells, as long-lived multicellular organisms depend on tissue replenishment of small pools of stem cells that must be self-renewed and maintained with a minimum of mutations throughout life. To ensure genome integrity, DNA must be replicated accurately and completely during S phase of the cell cycle. DNA replication initiates from numerous starting sites, so-called replication origins. Origins are licensed in large excess prior to DNA replication. When cells enter S phase, only a small subset of licensed origins are activated to initiate replication forks, leaving the rest to become dormant origins. Recently I have demonstrated that when primary replication forks are slowed or stalled, these dormant origins can initiate additional replication forks to promote complete genome replication. Without dormant origins, prolonged replication fork stalling will elicit fork collapse and chromosome recombination, which can result in genome rearrangement. Consistent with this idea, cultured human cells, C. elegans and mice partially depleted of dormant origins display significantly reduced survival when under replication stress. Furthermore, reducing dormant origins in mice by knocking down one of the licensing proteins resulted in stem cell deficiency and premature aging in mice. Therefore, we hypothesize that the use of dormant origin is a particularly important mechanism to ensure genome stability in stem cells. Due to the importance of genetic stability in human ES cells, particularly if they are to be used for medical applications, we propose to investigate the usage of dormant origins in hESCs and determine their role in maintaining hESCs genome integrity. As a comparison, we will also study dormant origins on cells at different stages during differentiation from hESCs into neural and hematopoietic lineages. To carry out this project, first we will study the overall process of origin licensing and DNA replication in hESCs and during their differentiation. This serves as a basis for our experiments at the next step where we will specifically compare dormant origin usage in hESCs and their derivative neural progenitor cells (NPC) and hematopoietic stem cells (HSC) when they are under replication stress (Aim 1). Based on my preliminary data on mouse ES cells, we expect hESCs employ dormant origins at high efficiency. Then we will determine the importance of dormant origin firing in maintaining genome stability in hESCs and their derivative NPCs and HSCs by experimentally modulating the number of licensed dormant origins and then examining the effect this has on the ability of cells to survive replication stress. In addition, we will investigate other possible mechanisms that contribute to hESCs genome stability such as DNA damage checkpoint and DNA repair (Aim 2). This will allow us to gain a systematic understanding of how hESCs maintain genome integrity. Finally we will determine the requirement of dormant origins during hESCs self-renewal and differentiation into various lineages (Aim 3). These experiments will reveal the physiological significance

of dormant origins and dissect their differential requirement during hESCs self-renewal and differentiation.

010SCA06

CT Stem Cell Research Proposal

Title of Project: **Nuclear Receptor Control of Human Epidermal Stem Cells**
Amount requested: $199,894; Amount funded: $199,894
Principal Investigator**: Brian J. Aneskievich, Ph.D.**
Collaborator: **Kursad Turkesen, Ph.D**. (Consultant)
Institution: **University of Connecticut**

This Project's purpose is to improve chronic and acute human skin wound repair by studying epidermal stem cell responses to dietary and clinical ligands following their application to cells in laboratory petri dish model systems.

Project Summary

The outermost layer of human skin, the epidermis, protects us from external assaults, bacteria, abrasions, and drying of underlying tissue. It accomplishes this daunting task while being less than four-thousandths of an inch thick. Epidermal cells, known as keratinocytes (KCs) go through repeated rounds of replication and maturation to replace cells lost to "wear and tear" as well as to heal wounds. KC stem cells are a reservoir of replacement cells but are partially lost with age or wounds like third-degree burns. Ageing reduces overall skin health and healing; 70-80% of patients with bedsores are over 65 translating to 700,000-800,000 older adults annually. Chance of death after a large-scale burn increases significantly faster than the percent increase in damaged skin area. *New treatments of epidermal wounds based on improved replication of remaining KC stem cells are needed to address these health needs.* We found certain dietary and clinical compounds which act as *receptor* ligands enhance KC replication. We hypothesize the ligands promote symmetric division resulting in relatively more stem cells and thus more cells in the replicating reservoir. An alternative mechanism predicts it is the progeny cells of stem cells that are affected. Several biological assays and biochemical tests will be used to examine these potential mechanisms. Additional preliminary results indicate a key protein, known as ΔNp63, expressed in KC stem cells, is increased by these ligands serving as a possible molecular switch for carrying out their effect. ΔNp63 is related to but antagonistic towards the growth retarding protein known as p53, which has recently been implicated in increased efficiency of reprogramming induced pluripotent stem cells (iPS). Following control versus ligand conditions, we will examine i)KC stem cell replication, ii)maturation markers in progeny cells, iii)levels of stem cell markers, and iv)the proteins ΔNp63 controls that carry out its stem cell enhancement effect. Results from this study could have two-fold benefits from both enhanced replication of KC stem cells for wound repair and these cell's use in iPS studies. A patient's own epidermis can be grown in laboratory dishes and used for clinical treatment of bedsores, diabetic leg ulcers and burns but it takes too long – upwards of 4 weeks – to make it practical for widespread clinical use. The ligand-receptor effect we identified could dramatically shorten this time. Alternatively, the improved KC stem cell replication needed for healing might be achieved by applying the ligand directly to the wound site. Either approach could be a *translational medical benefit* from this project's basic research.

010SCA13

CT Stem Cell Research Proposal

Title of Project: **The role of epigenetic factor-HP1 in regulating human embryonic stem cell pluripotency and differentiation**
Amount requested: $200,000; Amount funded: $200,000
Principal Investigator**: Ee-Chun Cheng**
Institution: **Yale University**

This Project's purpose is to investigate the role of epigenetic factor-HP1 in regulating human embryonic stem cell pluripotency and differentiation.

Project Summary

Emerging data point to a key role for epigenetic mechanism in embryonic stem cell (ESC) self-renewal, pluripotency, and lineage-specific differentiation. Each cell in the adult body contains exactly the same DNA (the same genome). The difference between distinct cell types lies in the particular subset of genes which are active (ie, "expressed") in a given cell or tissue. In the nucleus of eukaryotic cells, DNA is extensively folded and compacted with different types of proteins, thus forming a dynamic structure called chromatin. Diverse biochemical modifications of chromatin occur during development. These modifications, called epigenetic modifications, are known to profoundly regulate gene expression patterns. Recent studies suggest that as cells develop towards specific fates, regions of their genome are 'closed-down' by various epigenetic modifications. That is, as distinct areas of a cell's genome are shut down, their developmental potential becomes increasingly limited. Deciphering the nature of these epigenetic instructions in embryonic stem cells will have important implications for their future therapeutic use. Here we propose to study the role of a key epigenetic factor, Heterochromatin protein 1 (HP1), in regulating human embryonic stem cell (hESC) self-renewal and differentiation. HP1 is one of the most fundamental chromatin-associated proteins. It is well known that HP1 is essential for gene silencing throughout evolution and has an important role in human cells. We hypothesize that HP1 silences a variety of differentiation promoting genes in hESCs, thereby maintaining pluripotency. If this is true, loss of HP1 would destabilize the fate of hESCs in a fundamental way. Therefore, we will test if disrupting HP1 activity in hESC leads to defects in self-renewal and, further, if such disruption results in cellular differentiation (ie, loss of pluripotency) (Aim 1). Also, since HP1 is an epigenetic factor and can conceivably affect many regions of the genome, we will conduct high-resolution whole-genome mapping in hESCs to determine precisely which genes are bound by HP1 and how their expressions are affected by such interaction (Aim 2). Finally, since HP1 also brings other chromatin modifiers or transcriptional repressors to the genome, we will perform co-immunoprecipitation assays to isolate molecular complexes containing HP1, and identify unknown members within these complexes (Aim 3). If the above-proposed aims are achieved, this study will further our understanding of epigenetic regulation of stem cell self-renewal and open a new territory for hESC research. Moreover, since a reduction in levels of HP1 family members is correlated to certain cancers, this study may also provide a novel insight into oncogenesis and its relation to stem cell biology.

010SCA16

CT Stem Cell Research Proposal

Title of Project: ***In Vivo* Evaluation of Human ES, IPS and Adult Brain Derived Neural Progentor Cell Transplantation and Migration Using MRI**
Amount requested: $200,000; Amount funded: $200,000
Principal Investigator**: Erik M. Shapiro, Ph.D. (lead) and Eleni A. Markakis, Ph.D., (Co-PI)**
Institution: **Yale University**

This Project's purpose is to study the transplantation of differentiated neural precursor cells, created from different origins, to determine which cells would be best for localized neural cell therapy.

Project Summary

Human neural progenitor cells show tremendous promise in the treatment of central nervous system disease, in both cell supportive and cell replacement strategies. These cells can now be generated from adult human brain tissue, human embryonic stem (hES) cells, and induced pluripotent stem (iPS) cells. It is unclear what effect cell origins has on neural progenitors generated in these different ways. In this work we will undertake the first comparative studies aimed at characterizing cells generated from these three sources and evaluating their suitability for use *in vivo*. Using methodologies honed in our previous CT Stem Cell Seed Grants, we will establish optimal growth/proliferation conditions for cells of adult, hES, and iPS origins, establish their limits of proliferation, optimize growth arrest and differentiation paradigms *in vitro*, and study cell migration characteristics *in vivo* for cells of all three origins.

010SCA21

CT Stem Cell Research Proposal

Title of Project: **Regulating Caspase Activity to Enhance Differentiation Efficiency of Human Embryonic Stem Cells**
Amount requested: $200,000; Amount funded: $200,000
Principal Investigator**: Xiaofang Wang, Ph.D., M.D.**
Institution: **University of Connecticut Health Center**

This Project's purpose is to study the role of caspase-3 in hES cell differentiation and to enhance the differentiation efficiency by regulating caspase-3 activity.

Project Summary

The clinical use of human embryonic stem cells (hESCs) involves expansion and differentiating them into desired lineages. However, the differentiation of hESCs to a specific lineage can often be hardly achieved at very high efficiency, which is a bottleneck for many differentiation protocols. For example, the rate of hemangioblast (precursors of both endothelial and hematopoietic cells) differentiated from hESCs is often less than 10%. We have recently reported that an important programmed cell death factor, caspase-3, also plays a central role in inducing anoikis (detachment-induced death) and differentiation of hESCs. Caspases have also been shown by others to be critical for development and stem cell differentiation. However, the role of caspases in hESC differentiation has not yet been carefully studied. In this proposal, we hypothesize that caspase activity is involved in lineage-specific differentiation of hESCs, and we can enhance the differentiation efficiency by regulating caspase activity. We will use hemangioblast differentiation as a model system for this project. Specifically, we will first determine the role of caspase-3 in hESC differentiation to hemangioblast lineage. We will use small-molecule inhibitors and/or the shRNA to knock down the activity of caspase-3, and check whether hESC differentiation is affected. We will next investigate the mechanism underlying caspase-3-regulated hESC differentiation into hemangioblast lineage. Finally, we will investigate whether regulating caspase-3 activity can promote hESC differentiation into hemangioblast by either enhancing or decreasing the caspase-3 activity in hESCs at certain stages of their differentiation. In summary, completion of the proposed study will allow us to achieve new insight into the mechanisms for lineage-specific differentiation of hESCs, and develop new strategies to enhance the efficiency of hESC differentiation into therapeutically suited cell types.

010SCA22

CT Stem Cell Research Proposal

Title of Project: **Identification and characterization of multipotent cell populations from human adipose tissue for application in regenerative therapies**
Amount requested: $200,000; Amount funded: $200,000
Principal Investigator**: Matthew S. Rodeheffer**
Institution: **Yale University**
Collaborator: **Dr. John Persing**

This Project's purpose is to isolate and characterize subpopulations of cells from human adipose tissue in effort to identify superior starting material for use in tissue engineering and regenerative medicine.

Project Summary

The field of regenerative medicine aims to develop protocols for therapeutic replacement of damaged or diseased tissues or organs by tissue engineering, stem cell therapy or other approaches. Adult-derived stem cells are a conceptually attractive tool for regenerative medicine and tissue engineering due to the potential of generating replacement tissues from the recipient's own cells, eliminating the possibility of rejection. Of the tissues being considered as a source for adult-derived stem cells adipose tissue is one of the most promising, due to the abundance of available tissue and the relative ease of tissue isolation. Adult-derived stem cells that are capable of limited differentiation into several cell types in cell culture have been isolated from unfractionated adipose tissue. However, human adipose tissue contains many different cell types – including preadipocytes, endothelial cells and immune cells - whose specific potential for use in regenerative medicine remain poorly characterized. We hypothesize that human adipose tissue harbors distinct multipotent cell populations that will provide improved starting material for tissue engineering. We propose two objectives to investigate the functions of distinct cell subpopulations from human adipose tissue. For the first we will isolate different subpopulations of cells from human adipose tissue based on differential expression of cell surface markers, as determined by fluorescence-activated cell sorting, and determine their ability to become bone, cartilage, muscle and fat cells in cell culture. The second will test the hypothesis that these distinct multipotent cell populations will provide better starting material for regenerative medicine by testing the ability of these cell populations to form adipose tissue in vivo. These studies will provide the nucleus for future proposals and collaborations to establish the full potential of adipose tissue-derived cells in regenerative medicine in a range of applications, from recovery of heart function after a heart attack to generation of bioengineering of replacement blood vessels or fat tissue for use in reconstructive surgery.

10SCA23

CT Stem Cell Research Proposal

Title of Project: **Efficient methodologies to generate customized anti-tumor effecter T cells from human embryonic stem cells (hES) and induced pluripotent stem cells (iPS) by TCRengineering**
Amount requested: $200,000; Amount funded: $200,000
Principal Investigator**: Arvind Chhabra**
Institution/Hospital/Company: **University of Connecticut Health Cener**
Collaborators: **Dr. Ren-He Xu, Dr. Jijay Mukherji, Dr. James S. Economou**

This Project's purpose is to develop efficient methodologies to generate customized anti-tumor effecter T cells from human embryonic stem cells (hES) and induced pluripotent stem cells (iPS) by TCRengineering approach, for an effective cancer immunotherapy.

Project Summary
Currently there are four therapeutic approaches to target cancer. These include surgery, chemotherapy, radiation therapy and immunotherapy. Although surgery in combination with radiation and chemotherapy has long been regarded as the only effective method to treat cancer patients, immune based anti-cancer therapy is a promising strategy to treat cancer. The remarkable success of vaccines against infectious agents (bacteria and viruses) demands the development of a similar therapeutic approach towards cancer.

Although tumor-associated antigens for different human cancers have been identified and multiple strategies have been developed to induce anti-tumor cytolyticT lymphocytes (CTL), overall success with current cancer immunotherapy approaches has been modest. Novel strategies are needed to make this approach more effective.

One of the key limitations hindering the development of an effective immune based cancer therapy is extremely low precursor frequency of anti-tumor CTL in most cancer patients. We believe that the immense self-renewal potential of human embryonic stem cells (hES) and induced pluripotent stem cells (iPS), can be utilized to develop an effective cancer immunotherapy. Of note, the functional specificity of T cells is controlled by the T cell receptor (TCR) they express. With TCRengineering technology, we can isolate the TCR from T cells specific for a given antigen and engineer normal human T cells to acquire desired functional specificity. Utilizing this approach, we have isolated a human melanoma (skin cancer) associated antigen specific TCR and generated customized melanoma antigen specific anti-tumor T cells (published in Journal of Immunology, 2008).

In our proposed study, we aim to utilize this transgenic TCR to engineer hES and iPS and differentiate them into T cells. We believe that with this approach, we can generate a reservoir of customized patient specific anti-tumor T cells, for effective cancer immunotherapy.

10SCA24

CT Stem Cell Research Proposal

Title of Project: **Novel roles of long non-coding RNAs in human embryonic stem cells**
Amount requested: $200,000; Amount funded: $200,000
Principal Investigator**: Li Yang**
Institution/Hospital/Company: **University of Connecticut Health Center**
Collaborators: **Ling-Ling Chen and Gordon G. Carmichael**

This Project's purpose is to understand the novel roles of long non-coding RNA's in human embryonic stem cell self-renewal and pluripotency.

Project Summary

Although over 90% of the human genome is transcribed, less than 2% encode proteins and the vast majority of the genome is transcribed into non-protein coding RNAs (Human Genome Sequencing Consortium 2004). In addition to well-known and constitutively expressed housekeeping RNAs and small regulatory RNAs, an expanding number of long non-coding RNAs (lncRNA, larger than 200 nucleotides) have been identified and implicated in a variety of regulatory processes, including mammalian development and severe diseases. Most recently, Chen and Carmichael reported that the *NEAT1* lncRNA plays a key role in gene regulation by the nuclear retention of a class of mRNAs. Importantly, *NEAT1* is strongly suppressed in human embryonic stem cells (hESCs) but is induced upon differentiation. The absence of *NEAT1* RNA in hESCs might in fact allow the nuclear export of the mRNA for the pluripotency factor LIN28 (Chen and Carmichael, *Mol. Cell*, 2009). I have recently performed Solexa deep-sequencing of poly(A) selected RNAs from H9 hESC line and HeLa cells, and identified a number of lncRNAs that are specifically transcribed in hESCs, including those have not yet been annotated or described in the literature. In the proposed study, I will focus on two interesting but poorly documented lncRNAs, *HESRG* (human embryonic stem cells related gene) and *MIAT* (myocardial infarction associated transcript). While *HESRG* is a stem cell specific transcript, *MIAT* is somewhat more broadly expressed and is associated with the genetic risk of myocardial infarction. The preliminary studies revealed that these two lncRNAs have distinct cellular localizations and transcription profiles in hESCs, suggesting their distinct functions in hESCs. I propose to use this Seed Grant to decipher the roles of *HESRG* and *MIAT* in hESCs. I will first characterize the transcription and splicing regulation of *HESRG* and *MIAT* in hESCs and during lineages specific differentiation. I will also identify their associated proteins to understand their distinct localizations that can be important for their functions. Finally, I will study roles of *HESRG* and *MIAT* in stem cell growth and differentiation. Taken together, these studies will not only hold the potential to provide the first demonstration of the biological function for hESCspecific lncRNAs, but will also provide insights into a number of novel lncRNAs (that we have identified) in hESCs pluripotency and self-renewal.

10SCA29

CT Stem Cell Research Proposal

Title of Project: **Generation of layer V pyramidal neurons from human embryonic stem cells (hESC)**
Amount requested: $199,945; Amount funded: $199,945
Principal Investigator**: Radmila Filipovic**
Institution/Hospital/Company: **University of Connecticut**
Collaborators: **Joseph LoTurco, Brady Maher**

This Project's purpose is to develop methodology for generation of layer v pyramidal neurons which will integrate into host brain upon transplantation.

Project Summary

This project is aimed to develop methodology for successful generation of subtypes of layer V pyramidal neurons from hESC, which functionally integrate into host brain. This approach could be useful in future for cell replacement therapy following degeneration of motor neurons in ALS. The mammalian cerebral neocortex is a complex six-layered structure, where pyramidal neurons of deep layers (DL) neurons are generated first, while upper layers (UL) neurons are generated last. Recently, this sequential generation has been partially recapitulated *in vitro* suggesting that sequential generation of neocortical neuronal types is intrinsically regulated (Gaspard et al., 2008, Eiraku et al., 2008). We propose to develop a reproducible method for obtaining layer V neurons from hESC, to transplant them into rat brain, and to determine their projections and synaptic integration. While UL neurons project only within the cortex(cortical projection neurons, CPN), DL neurons send their projections mostly away from cortex and some within cortex. Within layer V, there are two major classes of pyramidal neurons: type I [corticofugal neurons (CFuN) which project to superior colliculus, pons and spinal cord (corticospinal motoneurons, CSMN)] and type II [corticoprojection (CPN) neurons which project within cortex]. Studying generation of CSMN is clinically very relevant, since these cells and their projections degenerate in amyotrophic lateral sclerosis (ALS) and in spinal cord injury (Molyneax et al., 2007). In the first Aim we will determine timeline of generation of different sybtypes of layer V pyramidal neurons from hESC. Some of the genes important for UL or DL neurons in rodents are identified. While Tbr2, Cux1, Cux2, Svet1 and Satb2 are important for development and postmitotic specification of CPN neurons, Tbr1, Fezf2, Ctip2, and Sox5 are important for generation of CFuN neurons (Hevner et al, 2001; Arlotta et al, 2005; Molyneaux et al., 2005). Satb2 is present throughout the cortical layers, and represses Ctip2 expression in callosally projecting neurons (Leone et al., 2008). We will over-express genes Ctip2, Sox5, Tbr2, or Satb2 *in vitro* in neuronal progenitor cells derived from hESC and determine whether thesegenes alter timing of generation and electrophysiological properties of layer V neuronal subtypes. In the second Aim we will test functional integration of subtypes of layer V pyramidal neurons derived from hESC after grafting into rat brain, with whole-cell electrophysiology usingbrain channelrhodopsin-2 (ChR2) assisted circuit mapping in rat brain slice Electrophysiological experiments will be performed in collaboration with Dr. Brady Maher.

10SCA30

CT Stem Cell Research Proposal

Title of Project: **Molecular mechanisms of germ layer induction in human embryonic stem cells**
Amount requested: $200,000; Amount funded: $200,000
Principal Investigator**: Efrat Oron**
Institution/Hospital/Company: **Yale University**
Collaborator: **Caihong Qiu, PhD**—Technical Director of the hESC Core, Yale Stem Cell Center; **David Tuck, MD**-Assistant Professor, Department of Internal Medicine; **Sherman Weissman, MD** —Sterling Professor of Genetics, Co Director, Yale Center for Excellence in Genomic Sciences; **Yibing Qyang, PhD**—Instructor of Medicine, Section of Cardiovascular Medicine, Yale Stem Cell Center

This Project's purpose is to identify the regulatory mechanisms that control initial steps of germ layer specification in human embryonic stem cells.

Project Summary
The ability of embryonic stem (ES) cells to grow in culture and give rise to diverse cell lineages can be exploited to produce specific cell types for medicine and research. Differentiation of ES cells is a multi-step process which begins with the induction of primary germ layers – ectoderm, endoderm and mesoderm. Very little data regarding this process is currently available and the key molecular components that control first steps of differentiation remain unidentified. Consequently, it has been difficult to faithfully recapitulate sequential steps of lineage specification in tissue culture.

The goal of this project is to decipher the molecular mechanisms guiding germ layer induction. To do this we propose to use an *in vitro* ES differentiation system combined with a functional genomics approach. First, we will use high-throughput sequencing to generate accurate and sensitive transcription profiles of human ES cells undergoing embryoid body differentiation. Bioinformatics tools will be used to analyze the data and select genes that are expressed prior to and during the emergence of germ layers and likely regulate ES cell differentiation. Next, we will down-regulate the selected genes using shRNAs and evaluate the potential of shRNAtargeted ES cells to give rise to primary germ layers. Finally, positive hits will be further tested using direct differentiation assays.

These studies are critical for obtaining a better understanding of how different cell lineages can be generated from ES cells. The discoveries made could lead to novel therapeutic approaches to repair tissues damaged by injury or disease.

10SCA35

CT Stem Cell Research Proposal

Title of Project: **Maturation of human embryonic stem (hES) cell-derived cardiomyocytes *in vitro* using 3D engineered tissue model system**
Amount requested: $200,000; Amount funded: $200,000
Principal Investigator**: Eun Jung Lee, Ph.D.**
Institution/Hospital/Company: **Yale University**
Collaborator: **Yibing Qyang, Ph.D.**

This Project's purpose is to induce maturation and differentiation of human embryonic stem cell-derived cardiomyocytes *in vitro* by mechanical forces using novel 3D engineered tissue model systems.

Project Summary
As one of the limitations currently with hES cell-derived cardiomyocytes is their embryonic phenotype based on their size, organization and electric properties in 2D culture, it is essential to better understand the microenvironmental cues that regulate differentiation and maturation of hESC-derived cardiomyocytes for advancement in stem cell-based therapy. Traditional 2D cultures are not physiologic and lack of mechanical stimulation and biological cues from other cell types may be the causes of immature cardiomyocytes as cardiomyocytes exist in an environment with extreme dynamic changes of stress and strain. Therefore, this proposal describes of using unique 3D engineered tissue systems incorporating mechanical stimulation such as mechanical stretching and shear stress to evaluate hESC-derived cardiomyocyte maturation *in vitro.* The innovation of this proposal primarily relates to a novel approach for evaluating hESC-derived cardiomyocyte function *in vitro*, using two custom-designed 3D engineered cardiac tissue models. First is the engineered cardiac chamber that allows the direct assessment of cardiomyocyte functionality, force generation, and pressure-volume loops, and is completely novel in its design. Second is the novel perfusion system that allows the culture various cell types in the presence of a bulk flow field, which is also, to the best of our knowledge, the first demonstration of *in vitro* perfusion of a gel-based system to study the impact of fluid flow on cell growth, remodeling and microvascular formation. These engineered tissues represent a more realistic model of a natural ventricle than traditional 2D planar cell cultures or 3D scaffolds, yet provide a more precise level of experimental control than animal or patient studies, and allow long-term cultivation *in vitro*. The ability to study human cardiac cell differentiation in 3D engineered tissue offering physiologic environment with mechanical cues will not only help to elucidate mechanisms on how cardiomyocytes mature, which is not possible with 2D cultures or *in vivo*, but also will greatly advance in developing strategies for cardiac repair as this may help to elucidate how new myocardial cells can properly integrate into the adult myocardium followed by the cell-therapy.

10SCA36

CT Stem Cell Research Proposal

Title of Project: **Generation of a novel source of iPS cells for the treatment of osteoarthritis**
Amount requested: $200,000; Amount funded: $200,000
Principal Investigator**: Rosa M. Guzzo, Ph.D.**
Institution/Hospital/Company: **University of Connecticut Health Center**

This Project's purpose is to generate a novel source of inducible pluripotent cells (iPS) to repair cartilage damage.

Project Summary
Arthritis results from degeneration of the cartilage lining the joints and is the leading cause of disability in the United States. By 2020, it is projected that 60 million Americans will have arthritis. Since cartilage lacks the capacity for self-repair, current treatment options are limited to pain reduction and joint replacement surgery. Thus, the development of novel therapeutic strategies to repair cartilage damage will benefit a significant portion of Americans.

There is widespread hope that embryonic stem cells, which possess the ability to give rise to any tissue of the body, may be used to repair cartilage damage from osteoarthritis (OA). It is now possible to create human stem cells from adult tissue without the use of embryonic cells. These cells, known as induced pluripotent cells (iPS cells) are generated by forced activation of four genes into adult skin cells. These four genes produce "reprogramming" factors that revert adult cells to an embryonic state with the ability to give rise to any cell type. Several cell types other than skin cells have also been used to generate iPS cells, however one question that remains is whether iPS cells retain any "memory" of the original cell type. For example, iPS cells derived from cartilage tissue may preferentially differentiate into cartilage cells as compared to iPS cells derived from skin cells. Thus, the goal of our proposed studies is to develop a novel human iPS cell-based approach for the treatment of cartilage degeneration.

We hypothesize that human cartilage cells harvested from osteoarthritic joints will bear a superior capacity to yield iPS cell-derived cartilage cells suitable for cartilage repair as compared to reprogrammed skin (fibroblast) cells. Therefore, we propose to first generate a novel source of human iPS cells from diseased cartilage and compare its cartilage forming potential to fibroblast-derived iPS cells. We will subsequently evaluate the ability of iPS cell-derived cartilage cells to repair articular cartilage using our animal model of surgically induced OA. Our proposed studies are original because, 1) this will be the first demonstration that articular cartilage cells can be reprogrammed; and 2) we will also, for the first time, demonstrate that these iPS cells have the ability to delay or reverse the progression of osteoarthritis in a translational animal model of surgical-induced arthritis.

10SCA38

CT Stem Cell Research Proposal

Title of Project: **Efficient Gene Targeting in Human Embryonic Stem Cell via Recombineering Based Long Arm Targeting Vector**
Amount requested: $200,000; Amount funded: $200,000
Principal Investigator**: Chunsheng Dong**
Institution/Hospital/Company: **Yale University**

This Project's purpose is to set up a platform for highly efficient gene targeting in human embryonic stem cells.

Project Summary
Human embryonic stem cells (hESCs) have the properties of self-renewal and pluripotency, and which present an excellent model to understand the molecular mechanisms of mammalian early development. Gene targeting is one of the key approaches to study the function of individual gene. In mouse genome thousands of genes have been knocked out and analyzed in *vivo* over the past 20 years. However, hESCs cannot be used to study mutations *in vivo.* Thus, gene targeting in hESCs is a valuable approach to reveal gene function *in vitro.* Unlike mouse embryonic stem cells (mESCs), hESCs are not easily amenable to efficient gene targeting as some groups have reported, which make this approach a challenging and laborious process in hESCs. The chief reasons include a low recombination frequency as well as a cloning efficiency. It has clearly been shown by some groups that the frequency of homolog recombination increased dramatically after increasing the homolog arms of the targeting vector. The traditional way to construct targeting vector is laborious work since one has to find appropriate restriction digestion–ligation strategies to put together usually six to seven DNA fragments. Here we seek a more efficient way to generate long homolog arm (about 10k) targeting vector based on recombineering in *E. coli*. We also aim to improve the method of targeting vector delivery in hESCs as well as the cloning efficiency. Our goal is to set up a platform for highly efficient gene targeting in hESCs.

10SCA47

CT Stem Cell Research Proposal

Title of Project: **Discovering treatments to prevent neurodegeneration in Huntington's Disease using hESC and patient-derived iPSC**
Amount requested: $200,000; Amount funded: $200,000
Principal Investigator: **Carolyn Drazinic, M.D., Ph.D.**
Institution/Hospital/Company: **University of Connecticut**
Collaborator: **Xue-Jun Li, Ph.D., Ren-He Xu, M.D., Ph.D.**

This Project's purpose is to find a possible treatment for Huntington's disease, by using IPSC technology to form patient-derived neurons and testing small molecules for inhibition or reversal of the disease process.

Project Summary
Huntington's Disease (HD) is a rare but devastating illness with an autosomal dominant inheritance, and it has severe neurologic and psychiatric manifestations. Patients who are affected develop a slow, irreversible deterioration in their neuromuscular function, leading to early disability and eventually death from complications of the illness. From a neurological standpoint, patients initially develop with decreased coordination, progress to choreoform or "dance-like" spastic movements of their extremities, and in advanced stages become confined to wheelchairs and nursing facilities. From a psychiatric standpoint, patients often experience depression or anxiety, and they go on to develop frank difficulties in short-term memory and sequencing of simple tasks, that leads to job losses, irritability, impaired judgment, impulsivity, and ultimately dementia.

Although the gene responsible for this disorder, *huntingtin*, was identified in 1991, and clinical tests are widely available to detect trinucleotide repeat expansions in this gene that cause the disease, the function of the *huntingtin* gene still remains a mystery, and there is still no treatment available to prevent the inevitable progression of the disease. This project has the following objectives: 1) Make induced pluripotent stem cells (iPSCs) from fibroblasts from skin biopsies from patients with HD, using standard stem cell technology. 2) Create patient-derived neurons (PDNs) from the iPSCs, using differentiation protocols for specific populations of neurons, such as peripheral motor neurons and brain striatal neurons, implicated in the disease. 3) Compare global changes in gene expression in iPSCs and during the differentiation into neurons using microarray technology, for both HD patients and unaffected controls. 4) Generate inhibitory RNA towards the *huntingtin* (Htt) gene in hESC cell lines obtained from the UConn Stem Cell Core, and study its impact different stages of neuronal differentiation using microarrays.
5) Test a number of molecules for their influence on the mutant *huntingtin* cell phenotype in PDNs and in transduced hESCs, including inhibitory RNA, neuropsychiatric medications, and other small molecules available through research collaborators.

Any molecules identified in this project that inhibit or reverse the aggregation of mutant *huntingtin* protein in neurons will potentially lead to a breakthrough in the treatment of Huntington's disease. Successful molecules will be candidates for clinical treatment trials for HD patients in the future.

10SCB02

CT Stem Cell Research Proposal

Title of Project: **Co-differentiation of hESC-derived retinal and retinal pigment epithelial progenitors**
Amount requested: $832,608; Amount funded: $832,608
Principal Investigator**: Lawrence J. Rizzolo**
Institution/Hospital/Company: **Yale University**
Collaborator: **Caihong Qiu, Co-PI**

This Project's purpose is to explore the use of human embryonic stem cells to treat diseases of the retina.

Project Summary

Many retinal diseases involve the retinal pigment epithelium (RPE) and its function as the outer blood-retinal barrier, either as a primary cause or a secondary consequence. The techniques for transplanting RPE and retinal cells are well established, but RPE transplantation usually fails, because the transplanted cells are not fully functional. We need to know more about how to produce the best cells for transplantation. This proposal investigates human embryonic stem cells as a source of RPE and retinal cells, and how to promote their maturation in culture in a way that improves their success in transplants. The investigation of the maturation process in culture may also lead to medical therapies that avoid transplantation altogether.

10SCB03

CT Stem Cell Research Proposal

Title of Project: **Use of human embryonic stem cells and inducible pluripotent stem cells to study megakaryoblastic leukemia**
Amount Requested: $1,000,000; Amount Funded: $1,000,000
Principal Investigator**: Diane Krause**
Institution/Hospital/Company: **Yale University**

This Project's purpose is to determine the cause of acute leukemia in infants.

Project Summary

The longterm goals of the research proposed are to determine how infantile leukemia develops. Some children are born with or diagnosed with leukemia within the first few months of life. In these children, the leukemia actually begins while the baby is still in utero from a population of blood stem cells that is no longer present in the same form after birth. Therefore, in order to study this leukemia, we propose to use human embryonic stem cells, which recapitulate this early fetal stage of blood development. Specifically, we will modulate these blood progenitors in order to determine how they become leukemia cells. In addition to helping to develop improved treatments for infantile leukemia, this work will also elucidate much about normal fetal and adult blood cell development, which can help to guide the development of novel therapies for adult leukemia and other blood diseases that cause hemorrhage, anemia, and infection.

10SCB05

CT Stem Cell Research Proposal

Title of Project: **Mechanical control of neural stem cell fate**
Amount Requested: $947,975; Amount Funded: $947,975
Principal Investigator**: Angelique Bordey**
Institution/Hospital/Company: **Yale University School of Medicine**

This Project's purpose is to examine the effects of mechanical cues on the proliferation, fate, translational profile of adult *neural stem cells*.

Project Summary

The adult human brain has been revealed to possess its own stem cells and is more plastic than we could have ever dreamed. Using neural stem cells (NSCs) to restore damaged or dying neurons or glia and improve brain functions in the cases of neurodegenerative diseases, stroke, and even aging is now plausible. However, the lack of markers of adult NSCs has significantly impended progress towards understanding the biology of these cells and their use for endogenous repair. In the adult human brain NSCs reside in the subventricular zone (SVZ) along the lateral ventricle and constitute a subpopulation (~5%) of cells expressing glial fibrillary acidic protein (GFAP). In aim I, we propose to identify which of the GFAP-cells behave as NSCs and develop approaches for studying them live in their microenvironment. Many cues in the local tissue microenvironment regulate the commitment of stem cells to different lineages. A recent literature suggests that mechanical stresses are important to regulate stem cell proliferation and fate. In the brain, hemodynamic forces exerted by blood flow exert cyclic strain on endothelial cells. Our preliminary data suggest that capillary constriction occurs essentially in the SVZ (and not outside the neurogenic niche) and leads to repetitive, transient mechanical stress in the NSC microenvironment. However the function of this mechanical stress is unknown. In Aim II, we thus propose that mechanical cues experienced in stem cell niche control the proliferation and fate of stem cells through specific changes in their translational profile. We will use a combination of techniques such as in vivo electroporation, imaging, and translational profiling to address our aims in mice and validate NSC markers in human tissue.

10SCB12

CT Stem Cell Research Proposal

Title of Project: **Generation of hematopoietic stem cells and T-cell progenitors from human embryonic stem cells**
Amount requested: $1,000,000; Amount funded: $1,000,000
Principal Investigator**: Laijun Lai**
Institution/Hospital/Company: **University of Connecticut Health Center**
Collaborators: **Irving Goldschneider** (Co-Investigator)

This Project's purpose is to induce human ES (and iPS) cells to differentiate in vitro into blood-forming (hematopoietic) stem cells and lymphocyte (T cell) progenitors that can reconstitute the lymphoid hematopoietic systems of immunodeficient mice (and ultimately human).

Project Summary

Hematopoietic stem cell transplantation (HSCT) is widely used in the treatment of cancer, aplastic anemia, complications of irradiation and chemotherapy, and autoimmunity. Bone marrow, umbilical cord blood (UCB), and mobilized peripheral blood cells are the major sources of HSCs. However, especially in adult patients, HSCT is frequently limited by the inability to obtain sufficient freshly harvested HSCs and to reliably expand them in vitro.

T-cells play a central role in the immune system by protecting against bacterial, viral, fungal and parasitic infections, and cancer. T-cell deficiency occurs under a number of circumstances, including aging, various genetic diseases, cancer, and infectious diseases such as AIDS. In addition, the generation of T cells after HSCT often is slow and incomplete, again resulting in life-threatening immunodeficiency.

Therefore, development of alternative sources of HSCs and T-cell progenitors remains an important goal that we will pursue in the present proposal by using human embryonic stem cells (hESCs) and induced pluripotent stem (iPS) cells.

10SCB19

CT Stem Cell Research Proposal

Title of Project: **Regulations of Lin28 in Human Embryonic Stem Cell Self-renewal and Differentiation**
Amount Requested: $1,000,000; Amount Funded: $750,000
Principal Investigator**: Caihong Qiu**
Institution/Hospital/Company: **Yale University School of Medicine**

This Project's purpose is to demonstrate the functions of Lin28 in human embryonic stem cell self-renewal and differentiation.

Project Summary

This proposal focuses on investigating the interaction of pluropotent factors to maintain human embryonic stem cells (hESCs) at their continuously renewing themselves state and not proceeding to more specialized cell types. We will specifically emphasize on the mechanistic study on how Lin28 positively regulates the Oct4 expression at the translational level, and how Oct4 stimulates the Lin28 expression.

Oct4 is a key component of molecular circuitry in regulating hESC growing and keeping their own property. Lin28, abundantly expressed in hESCs and directly regulating Oct4 gene expression, should play an important role in keeping hESCs in their unlimited growing and the ability to form other mature cell types. Also both Oct4 and Lin28 are among the four factors used to convert fibroblasts into molecularly and morphologically hESC-like cells, called induced pluripotent stem cells (iPSCs). The investigation of the mechanistic interaction between Oct4 and Lin28 will further illustrate the detail progression of converting fibroblasts to iPSCs, and their characteristics of hESCs and iPSCs. It will harness the clinical applications of hESCs and iPSCs.

We will also extend our scope of this proposal to investigate the biological function of Lin28 in the process of hESCs renewing themselves and changing themselves into other specified cell types. We will also work on identifying the new targets regulated by Lin28 in hESCs. Further exploring the roles of Lin28 will have a high impact on the therapeutic potential of hESCs and iPSCs. The success of this proposal will also add another layer of mechanistic understanding of hESC intra-regulation by translationally.

10SCB30

CT Stem Cell Research Proposal

Title of Project: **Modeling Parkinson's disease using human embryonic stem cells and patient-derived inducted pluripotent stem cells**
Amount requested: $992,500; Amount funded: $992,500
Principal Investigator**: James Yuanhao Li**
Institution/Hospital/Company: **University of Connecticut Health Center**
Collaborators: **Stormy Chamberlain, Eric Levine**

This Project's purpose is to develop an in vitro model of Parkinson's disease.

Project Summary

Parkinson's disease (PD) is the second most common neurodegenerative disorder. PD is characterized by loss of nigrostriatal dopaminergic neurons. The cause of the disease remains to be determined, and there is currently no cure for the disease. It is thus important to develop experimental models that recapitulate complete or key features of this human disease. Human genetic studies have recently identified genes linked to rare familial forms of PD. These findings have provided important clues in understanding the molecular and cellular pathogenesis of PD. Unfortunately, mouse mutants carrying mutations of these PD-associated genes fail to recapitulate key aspects of PD, such as a selective loss of nigrostriatal dopaminergic neurons. These findings suggest that there are human-specific aspects of PD. Therefore, it is imperative to develop an experimental model based on human cells, particularly human dopaminergic neurons. The overall goal of this proposal is to develop genetically modified human embryonic stem cells (hESCs) or derivation of PD patient specific induced pluripotent cells (iPSCs) and subsequent differentiation into dopaminergic neurons to provide in vitro models of PD. These cell-based models will provide clues for how PD develops, and be used to test for drug intervention.

10SCB36

CT Stem Cell Research Proposal

Title of Project: **Reconstitution of human hematopoietic system by human embryonic stem cells derived from human embryonic stem cells in humanized mice**
Amount Requested: $1,000,000; Amount Funded: $750,000
Principal Investigator**: Richard A. Flavell**
Institution/Hospital/Company: **Yale University**

This Project's purpose is to derive human hematopoietic stem cells from ES cells and verify their function in our mouse model of human immune reconstitution.

Project Summary

Our laboratory has developed a powerful system for the humanization of the immune system of mice, building on the Rag -/-γ C -/- immunodeficient mouse previously exploited by Traggiai et al. (Science 304:104-107(2004)). In brief, this involves engraftment of newborn mice with hematopoietic stem cells of human origin. In the original model, HSC from a variety of human sources have been shown to successfully engraft these mice including HSC from cord blood, fetal liver and adult. A major limitation of this system is a source of hematopoietic cells. Further it would be highly desirable to be able to match hematopoietic cells derived from a given human source with other tissue from that same source. Human ES cells provide a unique opportunity to generate HSC as well as other tissue and to repopulate the mouse in this way.

Engraftment of HSC in mice is still problematic and for that reason we have genetically modified mice at several loci to generate a unique recipient mouse which provides human factors which improve mouse engraftment by human HSC. In brief these factors include human cytokines, major histocompatibility complex molecules and other molecules that facilitate engraftment. However, a severe limitation of the utilization of this much improved mouse model is a source of human HSC of the kind listed above. We therefore propose in this grant to derive human HSC from human ES cells, to characterize these for their purity and stem cell capability and to engraft them into the genetically modified mice. Such engrafted mice will then be characterized for the efficiency of engraftment, fidelity of the lineages obtained and functionality of the cells.

10SCD01

CT Stem Cell Research Proposal

Title of Project: **Stem Cell Physiology and Chemistry Core**
Amount requested: $597,524; Amount funded: $500,000
Principal Investigator**: Srdjan D. Antic, M.D.**
Institution/Hospital/Company: **University of Connecticut Health Center**
Collaborators: Stormy Chamberlain, Eric Levine

This Project's purpose is to provide CT Stem Cell Grant Awardees with state-of-the-art equipment, expertise and service for physiological evaluation of stem-cell-derived cell lines of all types (blood, cardiovascular, muscle, bone and neuron), with special emphasis on the neuronal chemistry and physiology.

Project Summary

As result of the CT Stem Cell Initiative (2006-2010), we now have more than 20 laboratories in Connecticut that use human embryonic stem cells (hESc) or human induced pluripotent stem cells (iPSc) to generate colonies of mature human neurons. The critical step in this type of research is the evaluation of neuronal identity and functionality. It is now a very well established standard in the "stem-neuron" field that physiological maturation of differentiating neurons must be evaluated and confirmed using modern physiological assays (chiefly Patch-Clamp Recordings). Unfortunately, the greater majority of Connecticut stem-cell laboratories base their research solely on immunocytochemical and biochemical techniques.

Two factors have prevented those laboratories to pursue classical physiological recordings: (1) Lack of equipment; and (2) Lack of expertise. Here, I propose a complete solution to this problem. With a relatively modest financial support from the CT Stem Cell Initiative ($600,000), which less than the majority of previously awarded Principle grants, and far less than previously awarded Core grants, I can provide: (1) A fully equipped and functional CORE facility for carrying out the necessary measurements; (2) The expertise for several electrophysiological and chemical procedures; and (3) Trained personnel to execute these measurements in a systematic and uniform manner.

The use of the Physiology CORE facility has been envisioned to be cheap and affordable. Symbolic service fees (5 dollars per sample) would be charged to the CT Users. This is just an initial design. The rules of the CORE usage can easily be changed according to suggestions from the CT Dept. Public Health, or the Connecticut Stem Cell Research Advisory Committee, or UConn Health Center administration.

The central part of the proposed CORE is a Liquid Chromatography (LC) System for detecting nanomolar concentrations of substances in cultured cell lines. The proposed CORE will support detection of biological compounds in ALL stem cell-derived cell lines including: blood cells (*T-lymphocytes*), blood vessel wall and epithelia, heart muscle (*cardiomyocytes*), skeletal muscle (*myocytes*), bone (*osteoblasts*) and neuronal lineages (e.g. *forebrain neurons*, *dopaminergic neurons*, *spinal cord motoneurons*). LC System is relatively expensive (~$65,000), complex, and it requires specialized training. Within the proposed CORE this invaluable research tool may become immediately available to all CT Stem Cell Awardees.